Phenotypic plasticity in desiccation physiology of closely related, range restricted and broadly distributed fruit fly species.

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Supporting information

Methods

Standard gas equations:

Converting LiCor reading obtained in ppm to $mlCO_2.h^{-1}$

= (LiCor reading in ppm ÷ 1000000) x flow rate (100 ml/min) x 60min

= LiCor reading x 0.006

Converting mmol/mol H_2O to mg $H_2O.h^{-1}$

= (LiCor reading in mm/m ÷1000) x (1000 mmol ÷ 22400ml) x (18mg H2O ÷ mmol) x flow rate (100ml/min) x 60 min/h

= LiCor reading x 4.82

Statistical analyses were performed on $mlCO_2.h^{-1}$ but graphs were drawn on $\mu lCO_2.h^{-1}$ (all values * 1000 to transform from $mlCO_2.h^{-1}$ to $\mu lCO_2.h^{-1}$).

Results

Best model selection:

Survival assays

Table S1. The best model to compare the basal response between the four species (as represented by the control treatment) included sex but excluded body mass and body water.

Model selection: All species basal Longevity						
	Variables					
	Sex	**Body mass**	**Body water**	**Sex*Body mass**	**Sex*Body water**	**AIC score**
Model1	☒	☒	☒			3353.7
Model2	☒	☒				3413.7
Model3	☒		☒			3375.8
Model4		☒	☒			3307.2
Model5	☒					3298.5
Model6		☒				3415.5
Model7			☒			3397.7

Table S2. The best model for both *C. capitata* based on the AIC scores contained body mass and treatment and excluded sex and body water as predictors

Model selection: *C. capitata* Longevity					
	Variables				
	Treatment	**Body mass**	**Body water**	**Sex**	**AIC score**
Model1	☒	☒	☒		2265.7
Model2	☒	☒			2167.5
Model3	☒		☒		2362.2
Model4		☒	☒		2310.1
Model5	☒				2469.5
Model6		☒			2399.1
Model7			☒		2477.7

For *C. cosyra* and *C. podocarpi*, the best models included body water and treatment as predictor variables but excluding sex and body mass (Table S4, Table S5).

Model selection: *C. cosyra* Longevity					
	Variables				
	Treatment	**Body mass**	**Body water**	**Sex**	**AIC score**
Model1	☒	☒	☒		1352.4
Model2	☒	☒			1483.1
Model3	☒		☒		1462.4
Model4		☒	☒		1671.3
Model5	☒				1220.8
Model6		☒			1642.9
Model7			☒		1625.8

For *C. cosyra* and *C. podocarpi*, the best models included body water and treatment as predictor variables but excluding sex and body mass (Table S4, Table S5).

Model selection: *C. podocarpi* Longevity

	Variables				
	Treatment	**Body mass**	**Body water**	**Sex**	**AIC score**
Model1	✓	✓	✓		2372.5
Model2	✓	✓			2351.8
Model3	✓		✓		2130.6
Model4		✓	✓		2253.5
Model5	✓				2217
Model6		✓			2431
Model7			✓		2362.6

Table S3. The best model for *C. rosa* based on AIC scores contained body mass and treatment and excluded sex and body water as predictors.

Model selection: *C. rosa* Longevity					
	Variables				
	Treatment	**Body mass**	**Body water**	**Sex**	**AIC score**
Model1	☒	☒	☒		2474.5
Model2	☒	☒			2386.8
Model3	☒		☒		2230.6
Model4		☒	☒		2253.5
Model5	☒				2617
Model6		☒			2421
Model7			☒		2362.6

Table S6. For all species tested, $\dot{V}CO_2$ correlated positively with body mass so that heavier individuals had higher metabolic rates.

Body mass vs $\dot{V}CO_2$, Pearson's correlation				
Species	**t**	**df**	**p-value**	**R^2**
C. capitata	12,92	63	<0,001	0,85
C. cosyra	3,54	49	<0,001	0,45
C. podocarpi	10,25	43	<0,001	0,84
C. rosa	12,71	44	<0,001	0,89

Critical mass as predictor in survival assay

Metabolic and water loss measurements

Table S7. Model assumptions tested for all metabolic rate and water loss variables.

Normality shapiro	Capitata		Log(Capitata)		Rosa		Log(Rosa)		Cosyra		Log(Cosyra)	
	p	W	p	W	p	W	p	W	p	W	p	W
Body mass before (mg)	0,0005	0,9209	0,0005	0,9209	**0,2260**	0,9676	NA	NA	**0,5347**	0,9799	NA	NA
Body mass after (mg)	0,0035	0,9400	0,0035	0,9400	**0,1337**	0,9617	NA	NA	**0,2080**	0,9694	NA	NA
Mean body mass (mg)	0,0014	0,9315	0,0014	0,9315	**0,1764**	0,9648	NA	NA	**0,3249**	0,9741	NA	NA
Excretion events (n)	0,0000	0,5852	0,0000	0,5891	0,0000	0,6961	0,0000	0,5891	0,0000	0,6740	0,0000	0,6740
Excretion volume (ml)	0,0000	0,4598	0,0000	0,5908	0,0000	0,3565	0,0000	0,5908	0,0000	0,5174	0,0000	0,5174
Time spent active (%)	0,0000	0,8109	**0,7729**	0,9878	0,0062	0,9263	**0,7729**	0,9878	0,0001	0,8806	0,0001	0,8806
Metabolic rate (ml/h)	0,0002	0,9107	0,0000	0,8868	0,0007	0,8982	0,0000	0,8868	0,0003	0,8940	0,0003	0,8940
Resting metabolic rate (ml/h)	0,0002	0,9088	0,0000	0,8860	0,0068	0,9273	0,0000	0,8860	0,0002	0,8883	0,0002	0,8883
Lowest metabolic rate (ml/h)	0,0014	0,9315	0,0000	0,8792	0,0003	0,8862	0,0000	0,8792	0,0000	0,8628	0,0000	0,8628
Total water loss rate (mg/h)	0,0000	0,8335	0,0182	0,9547	0,0000	0,8126	0,0182	0,9547	0,0000	0,8581	0,0000	0,8581
Mean water loss rate (mg/h)	0,0000	0,6794	0,0000	0,8224	0,0000	0,8164	0,0000	0,8224	0,0001	0,8825	0,0001	0,8825
Lowest water loss rate (mg/h)	0,0000	0,8204	0,0258	0,9576	0,0000	0,8340	0,0258	0,9576	0,0001	0,8816	0,0001	0,8816
Cuticular water loss rate (mg/h)	0,0000	0,7830	**0,0771**	0,9667	0,0000	0,8011	**0,0771**	0,9667	0,0000	0,8578	0,0000	0,8578
Respiratory water loss rate(mg/h)	0,0000	0,8421	0,0219	0,9562	0,0000	0,7749	0,0219	0,9562	0,0000	0,8426	0,0000	0,8426
Gravimetric water loss rate (mg)	0,0000	0,8377	**0,5833**	0,9844	0,0001	0,8723	0,0000	0,5891	0,0000	0,7718	0,0000	0,7718

Body mass correlations

Mass correction vs Mass as co-variate

Table 8. Comparison of different metabolic rate analyses. Metabolic rates were analysed by species and treatment by using the following approaches: 1) ANCOVA with treatment as predictor and body mass as a co-variate, 2) GLM with treatment as a predictor of mass-adjusted metabolic rate 3) GLM with treatment as predictor and metabolic rate scales as per metabolic rate scaled with allometric equation (973Mb0.856) as calculated by calculated in Lighton et al. (2001)

		Mass as co-variate		**Mass adjusted values**		**Metabolic scaling**	
Species	**Comparison**	**F-score**	**p-value**	**F-score**	**p-value**	**F-score**	**p-value**
C. capitata	Control*Desiccation	-0.392	0.919	-0.471	0.885	-0.385	0.922
	Control*Temperature	0.524	0.860	0.325	0.943	-0.007	1.000
	Desiccation*Temperature	0.936	0.620	0.839	0.680	0.404	0.914
C. cosyra	Control*Desiccation	-0.537	0.854	-0.477	0.882	-0.999	0.581
	Control*Temperature	-0.195	0.979	-0.179	0.982	-1.075	0.534
	Desiccation*Temperature	0.337	0.940	0.296	0.953	-0.040	0.999
C. podocarpi	Control*Desiccation	-3.267	**0.0062**	-3.323	**0.0050**	-3.308	**0.0054**
	Control*Temperature	-4.443	**<0.001**	-4.274	**<0.001**	-4.516	**<0.001**
	Desiccation*Temperature	-1.144	0.4931	-0.916	0.6332	-1.178	0.4727
C. rosa	Control*Desiccation	-0.325	0.943	0.101	0.994	-1.184	0.469
	Control*Temperature	0.054	0.998	0.101	0.974	-0.548	0.848
	Desiccation*Temperature	0.398	0.916	0.119	0.992	0.627	0.806

Water loss measurements

Table S9. Additional water loss measures. The critical water loss for each species in each treatment (amount of water lost at death) as well as Water loss rate during desiccation as adjusted by surface-area using Meeh's formulae (see Hadley, 1994). TBSA = $kW^{2/3}$, with k=9.83

		Body mass (mg)		Critical water loss (ul)		Critical water loss (% body water)	Water loss rate (mg.h-1)		Water loss rate scaled ()	
Species	**Treatment**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**Mean**	**SD**	**Mean**	**SD**
C. capitata	Control	9,28	1,34	7,89	1,25	85,00	0,47	0,32	23,50	16,39
	Desiccation	9,28	1,39	6,68	1,31	72,00	0,34	0,18	16,69	8,81
	Temperature	8,08	1,89	6,06	1,22	75,00	0,47	0,23	26,93	14,82
C. cosyra	Control	9,66	1,99	8,21	1,56	87,00	0,35	0,28	16,48	12,54
	Desiccation	8,84	2,41	7,51	1,98	85,00	0,23	0,19	12,13	9,95
	Temperature	8,25	1,71	7,02	1,62	83,00	0,33	0,23	17,95	13,51
C. podocarpi	Control	9,07	1,28	7,71	1,22	78,00	0,52	0,38	26,24	18,73
	Desiccation	9,34	1,66	7,94	1,58	75,00	0,44	0,19	22,07	9,88
	Temperature	8,69	2,72	7,38	2,50	72,00	0,68	0,13	37,36	11,04
C. rosa	Control	11,56	2,25	9,83	1,14	77,00	0,41	0,23	17,01	9,46
	Desiccation	9,60	2,37	8,16	1,98	69,00	0,09	0,06	4,47	2,87
	Temperature	10,17	2,59	8,65	2,01	75,00	0,21	0,21	9,22	8,24

Table S10. Correlations between body mass and body water.

Body mass vs $\dot{V}H_2O$, Pearson's correlation				
Species	**t**	**df**	**p-value**	**R^2**
C. capitata	-1.085	63	0.281	-1.35
C. cosyra	1.070	49	0.096	0.235
C. podocarpi	-0.284	43	0.777	-0.043
C. rosa	4.3427	44	<0,001	0.548